

3628-Pos Board B356**Revealing Transient Interactions Between Phosphatidylinositol-Specific Phospholipase C and Phosphatidylcholine-Rich Lipid Vesicles**Boqian Yang^{1,2}, Mary F. Roberts², Anne Gershenson¹.¹Department of Biochemistry and Molecular Biology, University of Massachusetts, Amherst, MA, USA, ²Department of Chemistry, Boston College, Chestnut Hill, MA, USA.

Using *Bacillus thuringiensis* phosphatidylinositol-specific phospholipase C (*BtPI-PLC*) as a model amphitropic protein, we are investigating how membrane structure and composition affect protein-membrane interactions. Previous work showed that *BtPI-PLC* specifically binds to phosphatidylcholine (PC)-rich membranes and preferentially interacts with unilamellar vesicles with high curvature. In this work, we monitored single fluorescently labeled *BtPI-PLC* proteins as they cycled on and off surface-tethered phosphatidylglycerol (PG)/PC small unilamellar vesicles (SUVs) using total internal reflection fluorescence (TIRF) microscopy. The residence times on vesicles along with vesicle size information, based on vesicle fluorescence intensity, reveal the time scales of protein-membrane interactions as well as the curvature dependence. *BtPI-PLC* residence times on SUVs average 300 ms, similar to published residence times (300–400 ms) for other amphitropic proteins that transiently interact with cell surfaces. The kinetics of *PI-PLC*/membrane interactions is well explained by a simple two state binding model with dissociation and association rate constants averaging 3 s^{-1} and $0.6\text{ }\mu\text{M}^{-1}\text{ s}^{-1}$ respectively. In addition fluorescence correlation spectroscopy (FCS) measurements indicate that introducing lipid packing defects PG/PC SUVs by incorporating low mole percentages of dioleoylglycerol (DOG) enhances *BtPI-PLC* binding to SUVs. By combining these single molecule fluorescence results with previous biophysical measurements and molecular dynamics simulations, we have developed a quantitative model showing how the bacterial virulence factor *BtPI-PLC* interacts with cell membranes in molecular detail.

3629-Pos Board B357**Interplay of Membrane Lipids Differentially Affects Lipid Binding of Phosphatidic Acid Effectors**Priya Putta¹, Johanna M. Rankenberg¹, Christa Testerink², Edgar E. Kooijman¹.¹Department of Biological Science, Kent State University, Kent, OH, USA, ²Section of Plant Physiology, University of Amsterdam, Amsterdam, Netherlands.

The interaction of phosphatidic acid (PA) with membrane proteins is responsible for a host of cellular functions. To date no PA specific binding domain has been identified. Instead, electrostatic and hydrophobic interactions are likely to work in tandem to regulate PA effector-PA binding. Electrostatic interactions with the PA headgroup are explained by the electrostatic-hydrogen bond switch model, whereas hydrophobic interactions are explained by the negative curvature of (unsaturated) PA. In order to shed light on PA-protein binding we study the interaction of PA with PA effectors in complex lipid mixture, not just phosphatidylcholine (PC) bilayers, using liposome binding assays. Previously we showed that PE differentially affects the binding of PA effectors. We extended this work to now show that the opposite effect is observed in the presence of lyso-phosphocholine (LPC). We also show that under the right conditions dioleoyl glycerol (DOG) stimulates binding to PA for a well-known and extensively characterized PA-binding protein. PA-effector-PA binding is thus significantly affected by the presence of other membrane lipids. These studies show the need to incorporate other membrane lipids when investigating the interaction of putative PA binding proteins with PA, thereby further our understanding of PA mediated signaling.

3630-Pos Board B358**The Protein that Held Back the Dye: Annexin's Effect on Membrane Permeability**

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The membrane is more than a barrier that protects the cell; composed of lipids and protein, the membrane is implicated in signaling, cell stability, and protein interactions. They must be able to respond to stressors that can affect these roles, and employ different membrane components for that purpose. Cholesterol is a common component to both monolayers of the eukaryotic plasma membrane, moving freely and relaying of information such as changes in lipid distribution. The annexin family of membrane-associated proteins constitutes two percent of eukaryotic proteins within the cell. Annexins interact with multiple binding partners including small molecules like Ca^{2+} , phospholipids, and other proteins that are often involved in membrane repair. To determine

how binding of annexin impacts the permeability of the membrane, carboxy-fluorescein (CF) release assays were performed. CF efflux from vesicles in the presence of annexin a5 without Ca^{2+} showed a slight decrease compared to the control of vesicles alone; however, with the addition of both annexin and Ca^{2+} , the signal decrease was greater. In order to observe the effects of both cholesterol and protein on permeability, CF studies were repeated on vesicles containing increasing mole fractions of cholesterol. Less CF was released from vesicles containing cholesterol, and an even greater decrease was observed with annexin and Ca^{2+} added. This suggests that in the presence of Ca^{2+} , annexin works to reduce the permeability of the membrane, especially for cholesterol-containing vesicles. In previous work with isothermal titration calorimetry (ITC) we also observed a change in the Ca^{2+} binding parameters and stoichiometry of annexin a5 in the presence of cholesterol-containing membranes. This combined data, leads us to hypothesize that through their calcium binding ability, annexins sense the distribution of lipids and help communicate changes in the membrane environment.

3631-Pos Board B359**Membrane Insertion Pathway of the Apoptotic Repressor Bcl-xL: How (DIS)Similar is it to that of Diphtheria Toxin T-Domain?**

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The anti-apoptotic repressor Bcl-xL inserts into the mitochondrial membrane as a supposed part of its physiological action. While the exact molecular mechanism of this transition is poorly understood, the structural similarity of the water-soluble state of Bcl-xL with that of the diphtheria toxin T-domain led to the suggestion that their membrane-insertion pathways will be similar as well. Here we test this hypothesis by applying an array of spectroscopic methods to characterize and compare conformational switching and membrane insertion of the two proteins. CD spectroscopy and thermal denaturation measurements indicate that, unlike the T-domain, Bcl-xL is resistant to acid-induced destabilization in solution. FRET measurements between donor-labeled protein and acceptor-labeled vesicles demonstrate that Bcl-xL undergoes reversible membrane association strongly modulated by the presence of anionic lipids. In contrast, initial stages of membrane action of the T-domain are largely lipid-independent, with anionic lipids playing a role only on the later stages of a multi-step insertion pathway. Site-selective attachment of environment-sensitive fluorophore NBD to the helical hairpin of Bcl-xL ($\alpha 5$ - $\alpha 6$) or the T-domain (TH8-TH9) reveals similarities in the topology of the inserted state, but not in the lipid-dependent kinetic regulation of the insertion transition. Taken together our results indicate that while Bcl-xL and the T-domain share structural similarities, their mode of conformational switching and membrane insertion pathways are distinctly different. We suggest that these variations reflect underlying physiological differences: while cellular entry of the toxin via endosomal pathway requires robust insertion of the T-domain, the apoptotic control through the action of Bcl-xL and other members of the Bcl-2 protein family involves multiple levels of regulation, including those modulated by changes in mitochondrial lipid composition. Supported by NIH GM-069783 (A.S.L.) and Fulbright-CONICYT (M.V.U.).

3632-Pos Board B360**Do Acidic Residues in TH8-TH9 Play a Role in Transmembrane Insertion of the Diphtheria Toxin T-Domain?**Chiranjib Ghatak¹, Mykola V. Rodnin¹, Karin Öjemalm², Aurora Holgado², Mauricio Vargas-Urbe¹, IngMarie Nilsson², Gunnar von Heijne², Alexey S. Ladokhin¹.¹KUMC, Kansas City, KS, USA, ²Stockholm University, Stockholm, Sweden.

The translocation (T)-domain plays a key role in the entry of diphtheria toxin into the cell, where it inserts into the endosomal membrane and transfers the catalytic domain into the cytosol in response to endosomal acidification. Protonation of the three acidic residues located in the hydrophobic helical hairpin TH8-TH9 (E349, D352 and E362) has been suggested to modulate transmembrane insertion of the T-domain. Here, we test this hypothesis by combining site-directed mutagenesis with assays that test the conformational switching and bilayer insertion in the context of either isolated helical fragments or that of the entire T-domain. The propensity of individual helices to adopt a transmembrane conformation, studied using translocon-assisted insertion, reveal that only the most hydrophobic helix TH8 has a marginally favorable free energy of insertion. The free energy for TH8-TH9 hairpin was more favorable, yet much lower than that for the entire protein, suggesting a cooperative effect for T-domain membrane insertion. While mutations of acidic residues had no effect on insertion of individual helices, they had an effect in the context of the entire protein. E.g., E362Q mutant labeled with the environment-sensitive fluorophore NBD in the middle of TH9 inserts more efficiently than